# Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

Kurt W. Kohn, MD, PhD
Scientist Emeritus
Laboratory of Molecular Pharmacology
Developmental Therapeutics Branch
National Cancer Institute
Bethesda, Maryland
kohnk@nih.gov

## CHAPTER 11

## The camptothecin story: From a "Happy" Chinese Tree.

In 1960, the National Cancer Institute (NCI) began a search for anticancer substances in extracts from plants and animals (so called "natural products"). That effort was added to the ongoing testing of large numbers of organic chemicals for anticancer activity. The work was being carried out under the auspices of the NCI's Cancer Chemotherapy National Service Center (CCNSC). Every substance tested in this system received an NSC number to code for it in the database, which had information about chemical structure, origin, and test results in animals and cancer cell lines.

By far the most important discoveries by the natural products effort were camptothecin and taxol, both of which were isolated from plant material by Monroe Wall and Mansukh Wani at the Research Triangle Institute (RTI) in North Carolina (Kohn and Pommier, 2000). Here we tell the story of camptothecin; the story of taxol is told in Chapter 12.

According to Wani, when he arrived at RTI 1962, there was nothing there except 4 walls, and it was only when the 5th 'Wall' joined him at RTI that things started to move. Wall and Wani worked together in a life-long collaboration that yielded some of the most important advances in the history of cancer chemotherapy (Figure 11.1).

Before coming to RTI, Monroe Wall had directed a program at the U. S. Department of Agriculture (USDA) in a search for plant materials that could be used as a starting point for the synthesis of cortisone, which was at the time in short supply. The plant extracts were also sent to NCI for testing against cancer in mice, and an extract from the Chinese tree *Camptotheca accuminata* (Figure 11.2) was found to have anti-cancer activity. The tree was known as Xi Shu, "Happy tree."

Anticancer search however did not fit in USDA's mandate, and Wall's desire to find the anticancer substance in those extracts had to wait a few years until he moved to RTI.

In 1963, Wall and Mani started with 20 kg of bark and wood from *Camptotheca accuminata*. They made extracts from the material and tested them for anticancer activity in mice. They tested the most active samples at each purification step. It was slow and painstaking. But by 1966, they had pure camptothecin and had determined its structure by x-ray diffraction (Figure 11.5) (Wall, 1966).



Figure 11.1. Monroe E. Wall (right) and Mansukh C. Wani (left), discoverers of camptothecin and taxol.

Figure 11.2. *Camptotheca accuminata* (Xi Shu, "Happy tree") in the Chengdu Botanical Garden - Chengdu, China. It is native near the warm humid stream banks in Southern China and Tibet. (*Public domain, Wikipedia commons.*)

### Camptothecin specifically inhibits topoisomerase I.

The first clue that camptothecin targets a topoisomerase-like enzyme was unknowingly obtained by Susan B. Horwitz in 1973 in an early observation at a time when topoisomerases had not yet been discovered (Horwitz and Horwitz, 1973) (Figure 11.3). That was years before the name "topoisomerase" was invented. She had exposed human cancer cells to camptothecin, a novel anticancer drug, and observed that the cell's DNA strands were broken by the drug.

When the drug was removed, the DNA strand breaks quickly reversed. It seemed that the drug caused repairable or reversible DNA strand breaks. However, there was an additional observation that was so bizarre that it was not mentioned in her paper, perhaps because the paper might then not have been accepted for publication.

About the same time, a similar finding in cultured cancer cells was independently reported by Ann Spataro and David Kessel (Spataro and Kessel, 1972). Also about the same time, Rajalakshmi and Sarma (Rajalakshmi and Sarma, 1973) reported that camptothecin broke DNA strands in the liver of treated rats and that the DNA was repaired surprisingly quickly. According to Dr. Silvio Parodi, who worked with D.S. Sarma under the supervision of Emmanuel Farber at Fels Research Institute in Philadelphia, they were looking at anti-neoplastic agents (especially of natural origin) for their potential carcinogenicity, testing for induction of chromosomal aberrations and

sister chromatid exchanges when they observed the unusual DNA breakage and repair by camptothecin.

A few months before publication of Susan's paper, I visited her laboratory, which was then led by Arthur Grollman at Albert Einstein Medical Center in The Bronx. I had been studying DNA strand breakage and repair by various anticancer drugs, and she therefore told me about her findings with camptothecin. I then asked how long it took for the strands to be repaired. After some hesitation, Arthur Grollman said that the repair was very fast, so fast, even in the cold, that they could not measure it. I asked how that could possibly be. After further hesitation, Arthur said he didn't know, but that maybe there was an enzyme right there by the breaks that resealed them immediately when the drug was removed. That speculation seemed so bizarre that I could not accept it. However, it turned out that Grollman's speculation was right on the mark, and the responsible enzyme was later identified as the then unknown topoisomerase I. Susan Horwitz had observed a new anticancer drug-induced mechanism of DNA breakage and repair that was to have major impact on cancer chemotherapy.



**Susan Band Horwitz,**

Figure 11.3. Susan Band Horwitz, working at Albert Einstein Medical Center, discovered that camptothecin produced rapidly reversible DNA breaks. She also discovered that anticancer drug Taxol blocked microtubules (Chapter 10).

In view of the early evidence that camptothecin caused DNA breaks and that inhibitors of topoisomerase II caused protein-linked DNA stand breaks (see Chapter 8), Leroy Liu and his colleagues tested camptothecin against topoisomerase II. They were surprised to find that there was no effect on topoisomerase II, but found that

camptothecin induced topoisomerase I to produce both DNA strand breaks and DNA-protein crosslinks (Hsiang et al., 1985; Hsiang and Liu, 1988). Indeed, Joe Covey and Christine Jaxel in my laboratory confirmed that camptothecin produces typical protein-linked DNA strand breaks (Covey et al., 1989). As Susan Horwitz and Arthur Grollman had surmised, a DNA-associated enzyme (later identified as topoisomerase I) rapidly reversed the strand breaks; they would have been amazed to know at the time that their postulated reversal enzyme also produced the breaks in the first place.

### *Topoisomerase I resolves the over- and under-twisted DNA during transcription and replication.*

Figure 10.1 illustrates the first of the cell's topological problems. As the paired DNA strands separate during transcription or replication, the DNA twists are pushed ahead and would become bunched up to an extent that strand-separation could not continue. In the case of transcription, there is an additional problem behind the bubble of separated strands. When the transcribed RNA emerges (diagram B in Figure 10.1), the complementary DNA strands re-associate, but there are not enough twist to make the stable one twist per 10 base-pairs (Pommier, 2013).

The problem is solved by type I topoisomerases that transiently cleave one DNA strand and allow the strands to swivel and remove the excessive or deficient twists as the DNA or RNA synthesis machinery marches on. After swiveling has removed the stress on the DNA helix, the topoisomerase rapidly reseals the break (Figure 2).



A. DNA replication

Increasing twist

B. Transcription

RNA

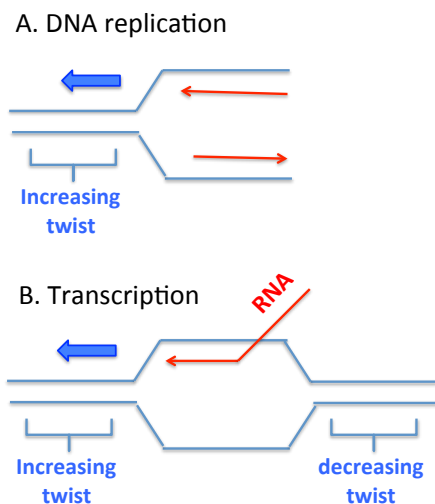Increasing twist        decreasing twist

Figure 10.1.  The DNA twisting problem in replication (DNA synthesis, A) and transcription (RNA synthesis, B). As the strands separate, the twists are pushed ahead and would impede further strand separation. The excessive or deficient

twists are resolved by topoisomerase I, which is bound to and moves along with the replication and transcription machineries. Parallel line pairs represent double-stranded helix. In A, the red lines represent newly synthesized DNA. In B, the red line represents newly synthesized RNA. The strand-separation forks are moving from right to left (fat blue arrows).
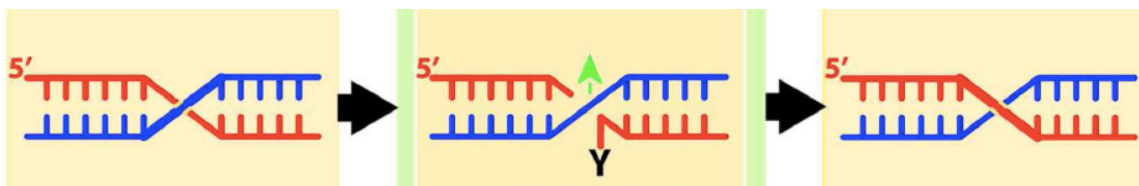


Figure 10.2. The topological problem resolved by a type 1 topoisomerases. The enzyme resolves the problem by breaking one DNA strand, allowing the other strand to pass through the break, and finally resealing the break. The red strand is broken and the blue strand passes through. As the enzyme breaks the strand, it grabs hold of one end via the enzyme's tyrosine (Y) at the active site (*(Pommier, 2013);  ACS Chemical Biology, permission needed*).

### How camptothecin causes DNA damage that kills cancer cells.

An early clue to how camptothecin kills cells was that it blocked the cell cycle in S phase, and the drug selectively killed cells when they were replicating their DNA (Goldwasser et al., 1996). We surmised that cells were selectively blocked in S phase because of collisions between moving replication forks and sites on the DNA where topoisomerase 1 was trapped by camptothecin (Figure 11.4). Our view was based on how topoisomerase I operates: it binds DNA in front of moving replication forks and cycles through opening and closing of a DNA strand break, so as to allow the strands to swivel and relieve the accumulating supercoiling of the DNA helix (Chapter 8, Figure 8.1). Camptothecin binds topoisomerase I while the latter is bound to DNA and has cut the DNA strand. When a moving replication fork collides with such a stabilized complex, it results in a free double-strand end that looks to the cell like a DNA double-strand break (Figure 11.4) (Kohn and Pommier, 2000).

We suspected that camptothecin stacked against DNA base pairs (as shown in Figure 11.4), based on experimental findings that were analogous to those we observed for topoisomerase II inhibitors; moreover, camptothecin preferentially trapped topoisomerase at sites where the enzyme linked to DNA at a G nucleotide (Chapter 8, Figure 8.8) (Jaxel et al., 1991).

Although cells actively replicating their DNA were most sensitive to camptothecin, absence of DNA replication did not fully preclude toxicity, probably because the RNA synthesis process ('transcription') could also collide with trapped camptothecin-topoisomerase-DNA complexes. Collisions due to progress of RNA synthesis,

however, produced a lesser degree of toxicity, perhaps because enzymes associated with the transcription machinery are able usually to clear the tracks for copying the DNA template strand..

More detailed investigation of the Top1-camptothecin interaction identified a mutant Top1 enzymes that had a change in the amino acid at a critical site in the protein. The mutant enzyme functioned as it should in relieving stressful DNA twists but did not produce DNA-Top1 trapped complexes with camptothecin. The point here is that cells having only this mutant form of Top1 were not killed by camptothecin. That finding was evidence that the trapped Top1-DNA complexes were in fact what was killing the cells, It was independent of the inhibition of strand untwisting, and therefore was attributable to the collision event *per se* (Andoh et al., 1987) (Gupta et al., 1988).

Thus, the very transient camptothecin-induced DNA breakage, originally observed by Susan Horwitz and Arthur Grollman, was found to be due to an effect on topoisomerase I (Top1) (Hsiang et al., 1985) (Covey et al., 1989). As with the topoisomerase II targeted drugs, DNA strand breaks and DNA-protein crosslinks were produced in equal numbers, consistent with one protein bound consistently to one end of each DNA strand break (Mattern et al., 1987). The covalent association of Top1 at each camptothecin-induced DNA break was then confirmed by Hsiang and Liu (Hsiang and Liu, 1988). Porter and Champoux then obtained evidence that the trapping of the Top1-DNA breaks was due to reduction by camptothecin of the rate at which the breaks reseal (Porter and Champoux, 1989). These studies established the essential features of how camptothecin traps DNA-Top1.

Later studies, however, disclosed that the formation of the disastrous DNA double-strand end shown in Figure 11.4 in cells treated with a topoisomerase inhibitor can be avoided if the drug concentration is not too high. When the growing end of a replicating DNA encounters a drug-induced block, the growing replication fork, instead of proceeding into the blocked region, can temporarily reverse, as shown in Figure 11.5 (Ray Chaudhuri et al., 2012). Figure 11.6 shows an electron microscope image of a reversed replication fork.

The new understanding in the 1980's of how camptothecin works greatly revived interest in testing the drug on cancer patients; camptothecin and related topoisomerase I inhibitors have since assumed an important role in cancer chemotherapy. The reversal of the replication fork is mediated in part by poly(ADPR) polymerase (PARP) (Ray Chaudhuri et al., 2012) (see Chapter 19).
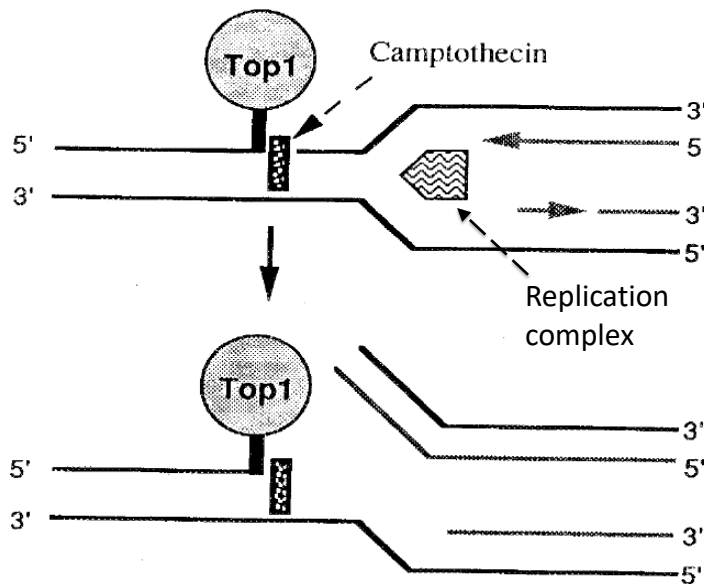
(Miao et al., 2006) – tdp1-scan1

Figure 11.4.  How an encounter between a moving replication process and a camptothecin-blocked topoisomerase I complex generates a potentially lethal DNA double-strand end, as we envisioned it in 1993 (Pommier et al., 1994). (Cells that are not in the process of replicating their DNA, however, are still somewhat sensitive to camptothecin, because of analogous encounters of trapped topoisomerase I by a  transcription process (Bendixen et al., 1990).)
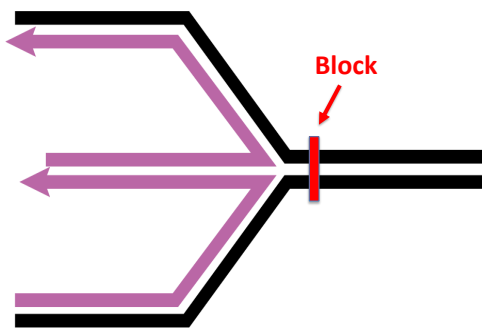


Figure 11.5. Reversal of replication fork when replicating DNA (brown lines) encounters a block, such as produced by a topoisomerase inhibitor. If the drug concentration is not too high, the replicating strands can reverse temporarily until the block is removed.
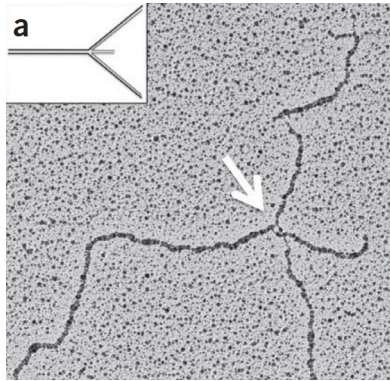
Figure 11.6. Electron microscope image of reversed replication fork (Ray Chaudhuri et al., 2012). Note the 4 DNA double-helices emerging from the reversal point (arrow). (*Permission needed.*)

### *Early clinical trials of camptothecin.*

As prelude to trials of camptothecin in cancer patients, testing in animals showed that a dose-limiting toxicity was damage to the lower intestinal tract. The cells of the inner lining of the intestines multiply rapidly in order to renew cells that normally are continually sluffed off. In cancer patients, however, the dose-limiting toxicity was suppression of blood cell production in the bone marrow (Gottlieb et al., 1970). Nevertheless, the rapidly dividing cells, both in the intestines and in the bone marrow, are particularly sensitive to camptothecin.

One of the problems with the early clinical trials of camptothecin was that they used the sodium salt form (Figure 11.7, right), which is inactive and its conversion to the active lactone form (left) in patients is erratic. The camptothecin lactone is the active form, but it is nearly insoluble and therefore difficult to prepare for clinical use. (The solubility problem was later solved by encapsulating the insoluble camptothecin lactone in gelatin capsules for oral administration).

However, the sodium salt is soluble and readily administered. It was therefore used in the early studies when its lack of activity was yet unknown (Muggia et al., 1996). The early clinical experience with camptothecin was discouraging, and therefore the clinical trials were stopped.

Camptothecin studies were resumed 15 years later when its action on topoisomerase I was discovered (Chapter 8). Development of camptothecin as an anticancer drug then resumed with renewed intensity, although the laps of 15 years was unfortunate for a drug that was to become very useful for anticancer therapy.
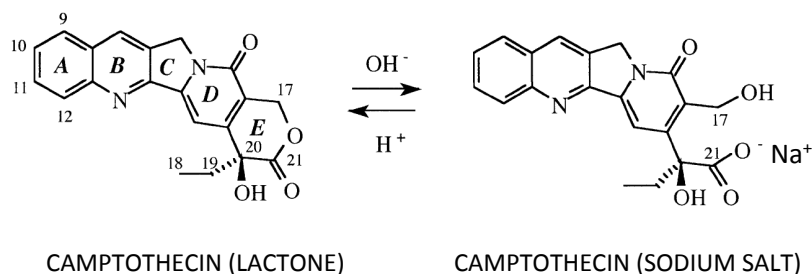
CAMPTOTHECIN (LACTONE)          CAMPTOTHECIN (SODIUM SALT)

Figure 11.7.  Chemical structure of camptothecin. The active form of camptothecin has a "lactone" structure in the E ring (left). Under alkaline conditions, the lactone ring opens to form the sodium salt (right), which is inactive. Under mild acidic conditions, the sodium salt slowly converts to the active lactone form. Notable also is that the natural active form has its OH group at position 20 pointing up, whereas the isomer whose OH points down is inactive. Thus the 3-dimensional structure around position 20 has to be just right for camptothecin to bind to the Top1 protein (see chapter 8).


***New drugs by modifying the structure of camptothecin.***

In 1989, we collaborated with Monroe Wall and Mansukh Wani in testing a large number of modified camptothecins for their activity against topoisomerase I (Jaxel et al., 1989) (Kohn and Pommier, 2000). The results showed where the camptothecin molecule could be modified to increase its potency and indicated where modifications abolished activity. We found out where the camptothecin molecule must remain unobstructed in order to fit into its binding site on the topoisomerase I protein, and where atoms could be added without loss of activity. For example, adding an NH2 group at position 9 on the A ring increased activity, whereas adding an NH2 group at position 12 destroyed activity. Thus, position 12 had to remain unobstructed to allow camptothecin to fit well into its binding site on topoisomerase I. Positions 10 and 11 were free for making small additions. In fact, adding an OH group, especially at position 10, substantially increased camptothecin potency (Jaxel et al., 1989).

Among the modified camptothecins we examined, one of the most potent had a methylenedioxy (-O-CH$_2$-O-) group added to form a 5-membered ring next to the A ring (Figure 11.8) (O'Connor et al., 1990; O'Connor et al., 1991). Although this compound was not pursued for development at that time, it was later rediscovered and called "FL118" (Ling et al., 2012; Ling et al., 2015).
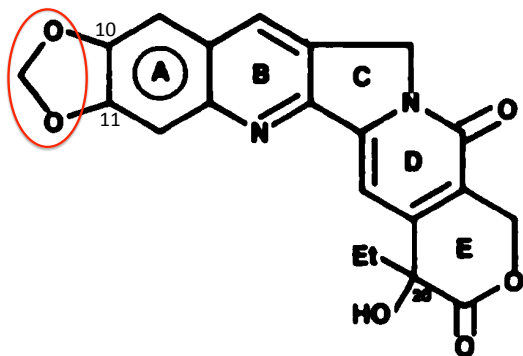
Figure 11.8. 10,11-methylenedioxycamptothecin, a modified camptothecin having increased potency for inhibition of topoisomerase 1 (Jaxel et al., 1989). The addition to the camptothecin molecule is circled red.

**Topotecan became the most frequently used camptothecin in cancer therapy.**

Our structure-activity findings (Jaxel et al., 1989) helped to design the modified camptothecin, "topotecan", which became commonly used in cancer treatment. Topotecan has a positively charged methylamino group added at position 9 and an OH group added at position 10 (Figure 11.9). The positively charged group solved the solubility problem; its placement at position 9 was in accord with our finding that additions could be made at this position without interfering with the ability of the drug to block topoisomerase I. We had also found that adding an OH group at position 10, which is the case for topotecan, would increase the potency of the drug. Topotecan was relatively easy to make by chemical modification of camptothecin, and it was highly potent against experimental tumors in animals, as well as effective against topoisomerase I in cancer cells (Kingsbury et al., 1991).
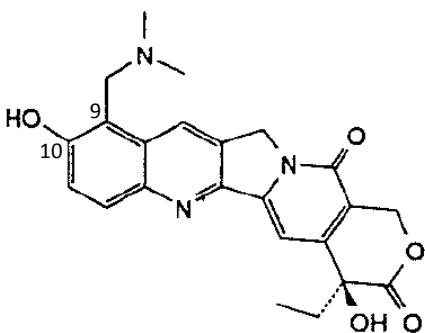


Figure 11.11.  Topotecan, a modified camptothecin became used in cancer therapy. The N-containing group added at position 9 becomes positively charged, and therefore improves the solubility of the drug, so that it can readily be administered to patients. The OH group added at position 10 increased the potency of the drug.

### *Irinotecan*

Another modified camptothecin, irinotecan, also became commonly used in therapy. It was approved by the U. S. Food and Drug Administration in 1996 for the treatment of colon cancer; it was also active against several other types of cancer. Irinotecan is a "pro-drug": it is nearly inactive until a carboxyesterase enzyme, present in liver and other tissues, cuts off an inactivating side-chain from the molecule (Figure 11.10A) (Ramesh et al., 2010).

When combined with other drugs, such as 5-fluorouracil and oxaliplatin, it became a key drug for the treatment of metastatic colorectal cancer, and it was also useful against several other types of cancer (Fujita et al., 2015).
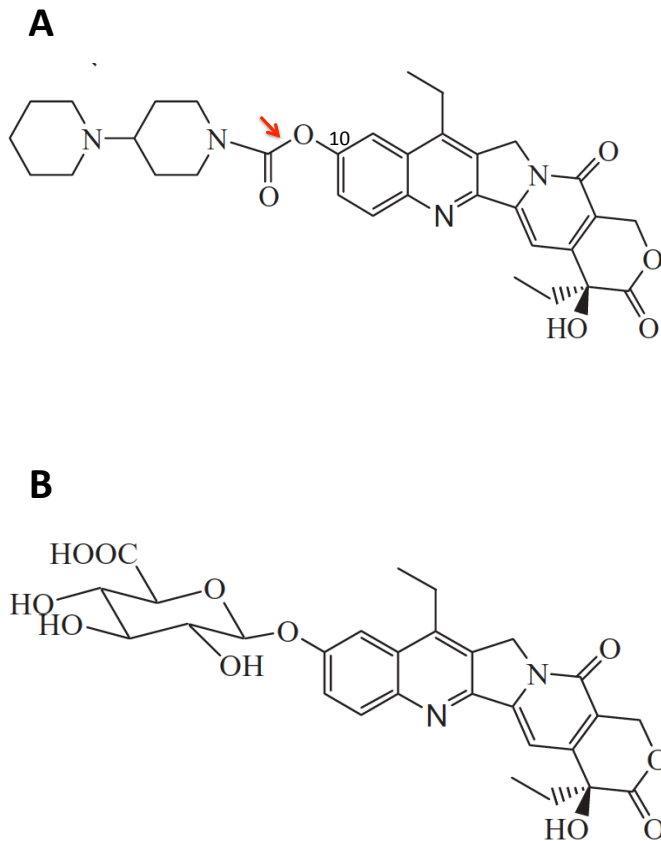
**A**



**B**



Figure 11.10. **A**. Chemical structure of irinotecan. The side chain on the oxygen at position 10 confers water-solubility, but inactivates the drug. The drug is activated by and enzyme present in tissues that cleaves of the bond indicated by the red arrow (Ramesh et al., 2010). That leaves an OH group on position10, which increases the potency of the drug (Jaxel et al., 1989).

      **B**. Chemical structure after addition of a glucose-like (glucuronide) unit by the UGT enzyme, which inactivates the drug (Ramesh et al., 2010). Absence of this enzyme allows the amount of available active drug to increase to higher levels and thereby makes patients who lack active UGT unusually sensitive to the drug.

Irinotecan produces unusually severe toxicity in some patients. Extensive studies were carried out to find out why that is the case. If the unusually sensitive patients could be identified, their drug dose could be reduced to a safe level. The studies revealed that a frequent cause of the unusual sensitivity was a particular isoform of a gene called UGT1A1 that sensitive patients had in their genome. This gene was found to code for an enzyme called UDP-glucuronosyl-transferase (UGT), whose function will be explained shortly. Among the several genes that code for UGT enzymes, the most troublesome form was UGT1A1*28. People who had only that isoform of the UGT1A1 gene were highly sensitive to irinotecan. The reason for that

was that the UGT enzyme made by that isoform was nearly inactive (Schulz et al., 2009) (Fujiwara and Minami, 2010).

According to Dr. Silvio Parodi, UGT (UDP-glucuronosyltransferase) is a cytosolic glycosyltransferase that catalyzes the transfer of the glucuronic acid component of UDP-glucuronic acid to a small hydrophobic molecule. This is a glucuronidation reaction. The reaction catalyzed by the UGT enzyme involves the addition of a glucuronic acid moiety to a variety of biologically active compounds found in nature.

To understand all that, we have to know what the active UGT enzyme does. After irinotecan has been activated by cutting off the side chain from position 10 (Figure 11.10A), UGT inactivates it again by adding a glucuronide unit (Figure 11.10B). Without active UGT, therefore, the level of active irinotecan was elevated to unusually high levels after the customary dose of the drug (Schulz et al., 2009) (Fujiwara and Minami, 2010). The solution to the irinotecan dosage problem therefore was to determine the UGT status of the patient and adjust the drug dosage accordingly.

A remarkable modification of irinotecan, called etirinotecan pegol, was designed that reduced toxicity and increased anti-tumor potency in mice by slowly releasing the active topoisomerase I inhibitor over long periods of time (Figure 11.11). The structure was designed to link irinotecan to long poly(ethylene glycol) chains in a manner that kept the drug inactive and to slowly and spontaneously release it in its active form (Hoch et al., 2014). Etirinotecan pegol was more effective than the bare irinotecan in suppressing the growth of tumors in mice (Figure 11.12), and clinical trials of this promising designer drug were begun (Alemany, 2014) (Jameson et al., 2013; Lopez-Miranda and Cortes, 2016).
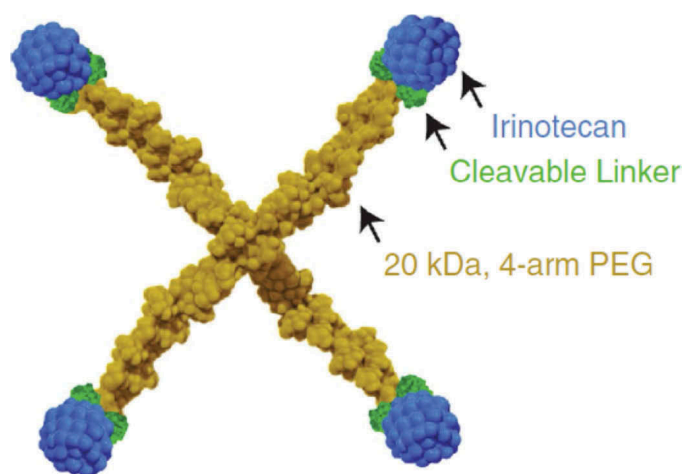
Figure 11.11. Molecular structure of etirinotecan pegol, in which irinotecan molecules were tethered to the ends of poly(ethylene glycol) chains. The linker slowly hydrolyses to release active irinotecan (Hoch et al., 2014) (*permission needed*).
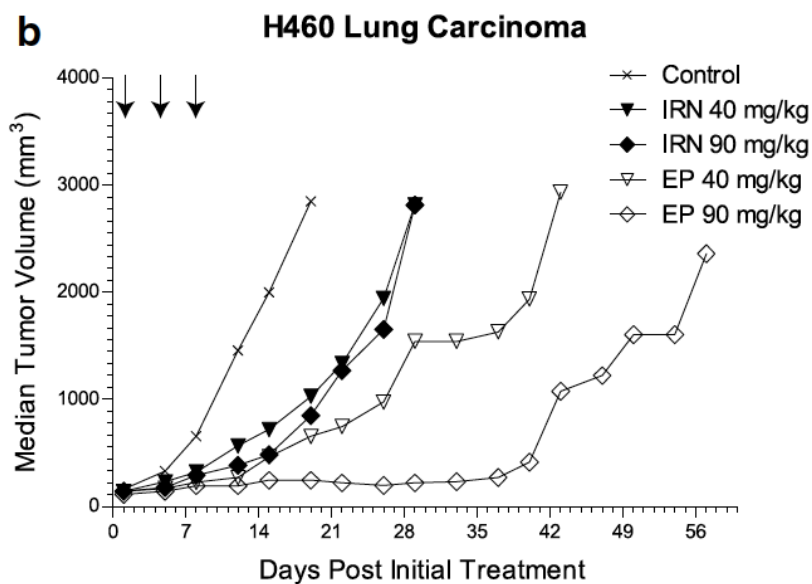


Figure 11.12. Increased effectiveness of etirinotecan pegol (EP) relative to bare irinotecan (IRN) against human lung cancer cells growing as tumors in immune-deficient mice (Hoch et al., 2014). Vertical axis: tumor volume; horizontal axis: time after treatment (arrows show times of EP injection). EP inhibited tumor growth for

a much longer time than did IRN. Similar results were reported with several cell lines from other types of human cancer. (*Permission needed.*)

Another way to make irinotecan more effective was to incorporate the drug in tiny microscopic-sized lipid globules called nanoliposomes, from which the drug was slowly released. In addition, the idea was that the nanoliposomes would be small enough to exit from the tumor's abnormal blood vessels, while being large enough to be retained in normal blood vessels. That would selectively deliver the drug to the tumor and reduce toxic effects to normal tissues. Another factor would be that drug within the tumor tissue would only slowly be flushed out, because of the poor lymphatic drainage that is common in tumors. Nanoliposomal irinotecan ("nal-irinotecan") has already been approved for clinical use (Ko, 2016).

## References

Alemany, C. (2014). Etirinotecan pegol: development of a novel conjugated topoisomerase I inhibitor. Current oncology reports *16*, 367.

Andoh, T., Ishii, K., Suzuki, Y., Ikegami, Y., Kusunoki, Y., Takemoto, Y., and Okada, K. (1987). Characterization of a mammalian mutant with a camptothecin-resistant DNA topoisomerase I. Proceedings of the National Academy of Sciences of the United States of America *84*, 5565-55611.

Bendixen, C., Thomsen, B., Alsner, J., and Westergaard, O. (1990). Camptothecin-stabilized topoisomerase I-DNA adducts cause premature termination of transcription. Biochemistry *29*, 5613-56111.

Covey, J.M., Jaxel, C., Kohn, K.W., and Pommier, Y. (1989). Protein-linked DNA strand breaks induced in mammalian cells by camptothecin, an inhibitor of topoisomerase I. Cancer research *49*, 5016-5022.

Fujita, K., Kubota, Y., Ishida, H., and Sasaki, Y. (2015). Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer. World journal of gastroenterology *21*, 12234-12248.

Fujiwara, Y., and Minami, H. (2010). An overview of the recent progress in irinotecan pharmacogenetics. Pharmacogenomics *11*, 391-406.

Goldwasser, F., Shimizu, T., Jackman, J., Hoki, Y., O'Connor, P.M., Kohn, K.W., and Pommier, Y. (1996). Correlations between S and G2 arrest and the cytotoxicity of camptothecin in human colon carcinoma cells. Cancer research *56*, 4430-4437.

Gottlieb, J.A., Guarino, A.M., Call, J.B., Oliverio, V.T., and Block, J.B. (1970). Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). Cancer chemotherapy reports Part 1 *54*, 461-470.

Gupta, R.S., Gupta, R., Eng, B., Lock, R.B., Ross, W.E., Hertzberg, R.P., Caranfa, M.J., and Johnson, R.K. (1988). Camptothecin-resistant mutants of Chinese hamster ovary cells containing a resistant form of topoisomerase I. Cancer research *48*, 6404-6410.

Hoch, U., Staschen, C.M., Johnson, R.K., and Eldon, M.A. (2014). Nonclinical pharmacokinetics and activity of etirinotecan pegol (NKTR-102), a long-acting

topoisomerase 1 inhibitor, in multiple cancer models. Cancer chemotherapy and pharmacology *74*, 1125-1137.

Horwitz, S.B., and Horwitz, M.S. (1973). Effects of camptothecin on the breakage and repair of DNA during the cell cycle. Cancer research *33*, 2834-2836.

Hsiang, Y.H., Hertzberg, R., Hecht, S., and Liu, L.F. (1985). Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. The Journal of biological chemistry *260*, 14873-14878.

Hsiang, Y.H., and Liu, L.F. (1988). Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. Cancer research *48*, 1722-1726.

Jameson, G.S., Hamm, J.T., Weiss, G.J., Alemany, C., Anthony, S., Basche, M., Ramanathan, R.K., Borad, M.J., Tibes, R., Cohn, A., *et al.* (2013). A multicenter, phase I, dose-escalation study to assess the safety, tolerability, and pharmacokinetics of etirinotecan pegol in patients with refractory solid tumors. Clinical cancer research : an official journal of the American Association for Cancer Research *19*, 268-278.

Jaxel, C., Capranico, G., Kerrigan, D., Kohn, K.W., and Pommier, Y. (1991). Effect of local DNA sequence on topoisomerase I cleavage in the presence or absence of camptothecin. The Journal of biological chemistry *266*, 20418-20423.

Jaxel, C., Kohn, K.W., Wani, M.C., Wall, M.E., and Pommier, Y. (1989). Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: evidence for a specific receptor site and a relation to antitumor activity. Cancer research *49*, 1465-14611.

Kingsbury, W.D., Boehm, J.C., Jakas, D.R., Holden, K.G., Hecht, S.M., Gallagher, G., Caranfa, M.J., McCabe, F.L., Faucette, L.F., Johnson, R.K., *et al.* (1991). Synthesis of water-soluble (aminoalkyl)camptothecin analogues: inhibition of topoisomerase I and antitumor activity. Journal of medicinal chemistry *34*, 98-107.

Ko, A.H. (2016). Nanomedicine developments in the treatment of metastatic pancreatic cancer: focus on nanoliposomal irinotecan. International journal of nanomedicine *11*, 1225-1235.

Kohn, K.W., and Pommier, Y. (2000). Molecular and biological determinants of the cytotoxic actions of camptothecins. Perspective for the development of new topoisomerase I inhibitors. Annals of the New York Academy of Sciences *922*, 11-26.

Ling, X., Cao, S., Cheng, Q., Keefe, J.T., Rustum, Y.M., and Li, F. (2012). A novel small molecule FL118 that selectively inhibits survivin, Mcl-1, XIAP and cIAP2 in a p53-independent manner, shows superior antitumor activity. PloS one *7*, e45571.

Ling, X., Liu, X., Zhong, K., Smith, N., Prey, J., and Li, F. (2015). FL118, a novel camptothecin analogue, overcomes irinotecan and topotecan resistance in human tumor xenograft models. American journal of translational research *7*, 1765-1781.

Lopez-Miranda, E., and Cortes, J. (2016). Etirinotecan pegol for the treatment of breast cancer. Expert opinion on pharmacotherapy *17*, 727-734.

Mattern, M.R., Mong, S.M., Bartus, H.F., Mirabelli, C.K., Crooke, S.T., and Johnson, R.K. (1987). Relationship between the intracellular effects of camptothecin and the inhibition of DNA topoisomerase I in cultured L1210 cells. Cancer research *47*, 1793-1798.

Miao, Z.H., Agama, K., Sordet, O., Povirk, L., Kohn, K.W., and Pommier, Y. (2006). Hereditary ataxia SCAN1 cells are defective for the repair of transcription-dependent topoisomerase I cleavage complexes. DNA repair *5*, 1489-1494.

Muggia, F.M., Dimery, I., and Arbuck, S.G. (1996). Camptothecin and its analogs. An overview of their potential in cancer therapeutics. Annals of the New York Academy of Sciences *803*, 213-223.

O'Connor, P.M., Kerrigan, D., Bertrand, R., Kohn, K.W., and Pommier, Y. (1990). 10,11-Methylenedioxycamptothecin, a topoisomerase I inhibitor of increased potency: DNA damage and correlation to cytotoxicity in human colon carcinoma (HT-29) cells. Cancer Commun *2*, 395-400.

O'Connor, P.M., Nieves-Neira, W., Kerrigan, D., Bertrand, R., Goldman, J., Kohn, K.W., and Pommier, Y. (1991). S-phase population analysis does not correlate with the cytotoxicity of camptothecin and 10,11-methylenedioxycamptothecin in human colon carcinoma HT-29 cells. Cancer Commun *3*, 233-240.

Pommier, Y., Leteurtre, F., Fesen, M.R., Fujimori, A., Bertrand, R., Solary, E., Kohlhagen, G., and Kohn, K.W. (1994). Cellular determinants of sensitivity and resistance to DNA topoisomerase inhibitors. Cancer investigation *12*, 530-542.

Porter, S.E., and Champoux, J.J. (1989). The basis for camptothecin enhancement of DNA breakage by eukaryotic topoisomerase I. Nucleic acids research *17*, 8521-8532.

Rajalakshmi, S., and Sarma, D.S. (1973). Rapid repair of hepatic DNA damage induced by camptothecin in the intact rat. Biochemical and biophysical research communications *53*, 1268-1272.

Ramesh, M., Ahlawat, P., and Srinivas, N.R. (2010). Irinotecan and its active metabolite, SN-38: review of bioanalytical methods and recent update from clinical pharmacology perspectives. Biomedical chromatography : BMC *24*, 104-123.

Ray Chaudhuri, A., Hashimoto, Y., Herrador, R., Neelsen, K.J., Fachinetti, D., Bermejo, R., Cocito, A., Costanzo, V., and Lopes, M. (2012). Topoisomerase I poisoning results in PARP-mediated replication fork reversal. Nat Struct Mol Biol *19*, 417-423.

Schulz, C., Boeck, S., Heinemann, V., and Stemmler, H.J. (2009). UGT1A1 genotyping: a predictor of irinotecan-associated side effects and drug efficacy? Anti-cancer drugs *20*, 867-8711.

Spataro, A., and Kessel, D. (1972). Studies on camptothecin-induced degradation and apparent reaggregation of DNA from L1210 cells. Biochemical and biophysical research communications *48*, 643-648.

Wall, M.E., Wani M. C., Cook, C. E., Palmer, K. H., McPhail, A. T., Sim, G. A. (1966). Plant Antitumor Agents. I. The Isolation and Structure of Camptothecin, a Novel Alkaloidal Leukemia and Tumor Inhibitor from Camptotheca acuminata. Journal of the American Chemical Society *88*, 3888.